Impact of Commercial Hatchery Practices on the Contribution of Broodstock to Offspring and Genetic Diversity in the Yellowtail Kingfish Breeding Program

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Abstract:

Using small numbers of broodstock for breeding often leads to random drift in gene frequencies between generations. Effective population size (N_e) is used as an indicator to assess the rate of inbreeding and loss of genetic variation in small populations. Genetic variation can be reduced further due to hatchery practices, such as pooled mating in broodstock groups with ramifications for long-term sustainability and management of inbreeding level in breeding programs. Pooled mating in groups generally results in differential contributions by broodstock, which can be compounded by differential larval/juvenile survival during metamorphosis and subsequent rearing. The level of genetic variation can be monitored by maintaining pedigree information. In this study we used six microsatellites, coupled with DNA parentage analyses (using PAPA 2.0), to determine the relative contributions from two commercial Yellowtail Kingfish (*Seriola lalandi*) hatchery broodstock groups over four pooled spawning events (two for each group). Up to three dams and five sires had the potential to participate in the pooled spawning. Initial broodstock contributions from the dams was found to be highly skewed, with one dominant dam contributing more than 70% of the offspring (1-day post hatch, dph) in three of the pooled spawning events, while two dams contributed fairly equally to the offspring in the fourth pooled spawning event. Two sires were found to be contributing more that 78% of the offspring in one pooled spawning event, while contributions between the sires were fairly equal in the other three spawning events. Over all spawning, N_e was less than half of the census N_e. We then examined the differential survival of family size through the hatchery production (from 1 to 25 dph) during which time mortality averaged 80%. Parentage analyses indicated that through the process some maternal and paternal contributions that had been initially represented in the 1-dph offspring

were lost. There were also significant changes in the frequencies of progeny from individual dams and sires from day 1 to day 25. These results indicate that hatchery breeding practices and high mortality during early larvae culture would result in poor retention of genetic variability across generations. With the actual N_e being less than half of the census N_e inbreeding rates would be relatively high if the current broodstock population was closed and future broodstock derived from existing hatchery systems.

1. Introduction:

The Yellowtail Kingfish (YTK), *Seriola lalandi*, is an important aquaculture finfish species in Australia, with an estimated annual production of 3,000 tons in 2008/09 at a value of AU$20 million. Current hatchery production is based on small numbers of wild caught broodstock although captive bred F_1 broodstock are becoming available to use as broodstock. As culture expands there is a growing interest in the domestication and genetic improvement of the species. Before embarking on a closed population-breeding program, the long-term sustainability of the mating system and the management of genetic variation and inbreeding needs to be actively considered.

Sustainability of a breeding program depends largely on the existing genetic variability in the founding population and its management across generations. If variability is low at the beginning or decreases across generations it can lead to inbreeding and decline in long-term response to selection (Norris *et al*., 2000 & Ditlecadet *et al*., 2006). Therefore capturing and preservation of genetic variability is of paramount importance in the design of domestication and breeding programs (Dupont-Nivet *et al*., 2006).

Since it is currently difficult to induce spawning by artificial means in YTK, natural or pooled mating in groups of broodfish is the commercial norm. Pooled mating often produces variable family sizes of unknown pedigree (Komen *et al*., 2006 & Castro *et al*., 2007) and skewed contributions from parents to offspring. Skewed contributions have been reported in other aquaculture species, such as Atlantic cod (Bekkvold, 2006; Rowe, 2007), and Nile tilapia (Fessehaye *et al*., 2006). Beside differential broodstock contribution at spawning time, differential larva/juvenile survival during metamorphosis and size grading are among the other potential factors, which may reduce genetic variation in hatchery populations.

During the commercial production of YTK larvae, generally three dams and four sires are placed for the summer spawning season in a 70-ton tank. Fish can spawn as frequently as every three days with each female producing one to two millions eggs and eggs are collected by skimming the day after an overnight spawning. The broodstock spawning activity is artificially induced through maintaining a summer photoperiod of 14 hours light: 8 hours dark). With this type of mating structure, it is impossible to identify the offspring of each parent and construct pedigrees for the offspring without the use of genetic markers. As a result there is no information on the impact of hatchery practices on family structure and management of genetic variability in closed populations of YTK.

The development of highly informative DNA markers makes it possible to construct pedigree and determine the genealogical relationships among offspring produced from pooled mating. Microsatellites have been extensively used to evaluate the impact of the natural mating and hatchery practices on the genetic diversity in aquaculture species, including Atlantic salmon *Salmo salar* (Norris *et al*., 2000), common carp *Cyprinus carpio* L. (Vandeputte *et al*., 2004), white sturgeon *Acipenser*

transmontanus (Rodzen *et al*., 2004), Senegal sole *Solea senegalensis* (Castro *et al*., 2006), gilthead seabream *Sparus aurata* (Castro *et al*., 2007), Atlantic cod *Gadus morhua* (Herlin *et al*., 2008) and silver carp *Hypophthalmichthys molitrix* (Gheyas *et al*., 2009).

Polymorphic microsatellite loci are available for the genus *Seriola* to be used for the parentage analysis (Nugroho and Taniguchi, 1999; Ohara *et al*., 2003; Renshaw *et al*., 2006; Renshaw *et al*., 2007). Recently Miller *et al*. (2010) has demonstrated the potential of these loci for the analysis of genetic variability and structure of wild and cultured stocks of YTK.

The objective of this study was to gain an insight into the impact of industry hatchery practices on the mating structure and management of genetic variability in closed hatchery populations with regard to the viability of future breeding programs in YTK.

2. Materials & Methods:

2. 1. Broodstock groups

This study involves two breeding groups that come from CleanSeas Aquaculture's Hatchery at Arno Bay, South Australia. Farm records showed that the broodstock (five sires and three dams) in the first breeding group were introduced into the hatchery in 2006 and 2008 and the broodstock in the second group (four sires and three dams) were introduced into the hatchery in 2008. All breeders were either coded wire tagged or PIT-tagged as a routine procedure and a fin tissue sample was taken for DNA analysis. The fin samples were stored in 99.9% ethanol at 4^0C until processed. The spawning activities of all broodstock were synchronized through

maintaining the photoperiod (14 hour light: 8 hours dark). Eggs were collected towards the end of spawning season and separately in two spawning events (one month apart from each spawning event) from both breeding groups and 200,000 eggs were randomly separated out in each spawning event for each breeding group and incubated separately.

2. 2. Larval Rearing

Fertilized eggs of Yellowtail Kingfish from two groups were obtained from hatchery and transported to the South Australian Aquatic Sciences Centre of the South Australian Research and Development Institute (SARDI), Adelaide. Upon arrival eggs from the two breeding groups were hatched in separate 120-l fiberglass incubators at 23 ^{0}C. After hatching, 2-dph fish larvae from the two breeding groups were stocked in separate 170-l fiberglass-rearing tanks at a stocking density of 100 larvae per liter with three replicates for each breeding group.

All rearing tanks were supplied with filtered seawater through a 5-μm filter in a flow-through system with a water exchange rate of 1.0 l/min at the beginning and increased to 2.0 l/min towards the end of the experiment to flush out the waste and excess food residues through overnight water exchange without food addition to the tank. Aeration was supplied through a single air stone to maintain dissolved oxygen at the saturation level and distribute the larvae and live foods evenly throughout the water column in the tank. A light regime of 13 h light: 11 h dark was provided with a light intensity at 800 to 2400 lux. Salinity was 38 ppt, the average salinity of YTK nursery grounds in South Australia (Chen *et al*., 2007). Throughout the experiment, the water temperature was maintained at 23 ± 0.5 ^{0}C (mean ± SD).

The larvae were then cultured from 2 to 25-dph, where all rearing tanks were fed rotifers (*Brachionus plicatilis*) from 3 to 12-dph. The rotifers were cultured with micro-algae (*Nannochloropsis oculata*) and enriched with S-presso (INVE Aquaculture) at 0.175g/l for 6 h before being added to the rearing tanks. *Artemia* nauplii enriched with DHA Selco (INVE Aquaculture) at 0.6 g/l for 12 h were introduced to the rearing tanks from 8 to 21-dph. Cyclop-EEZE a bio-engineered nutritional organisms (Argent Chemical Laboratories) was mixed with commercial compound diet (Otohime B1, Marubeni Nisshin) and supplied to the larvae from 16 to 22-dph. The larvae were supplied with the commercial compound diet (Otohime B2, Marubeni Nisshin) from 23 to 25-dph.

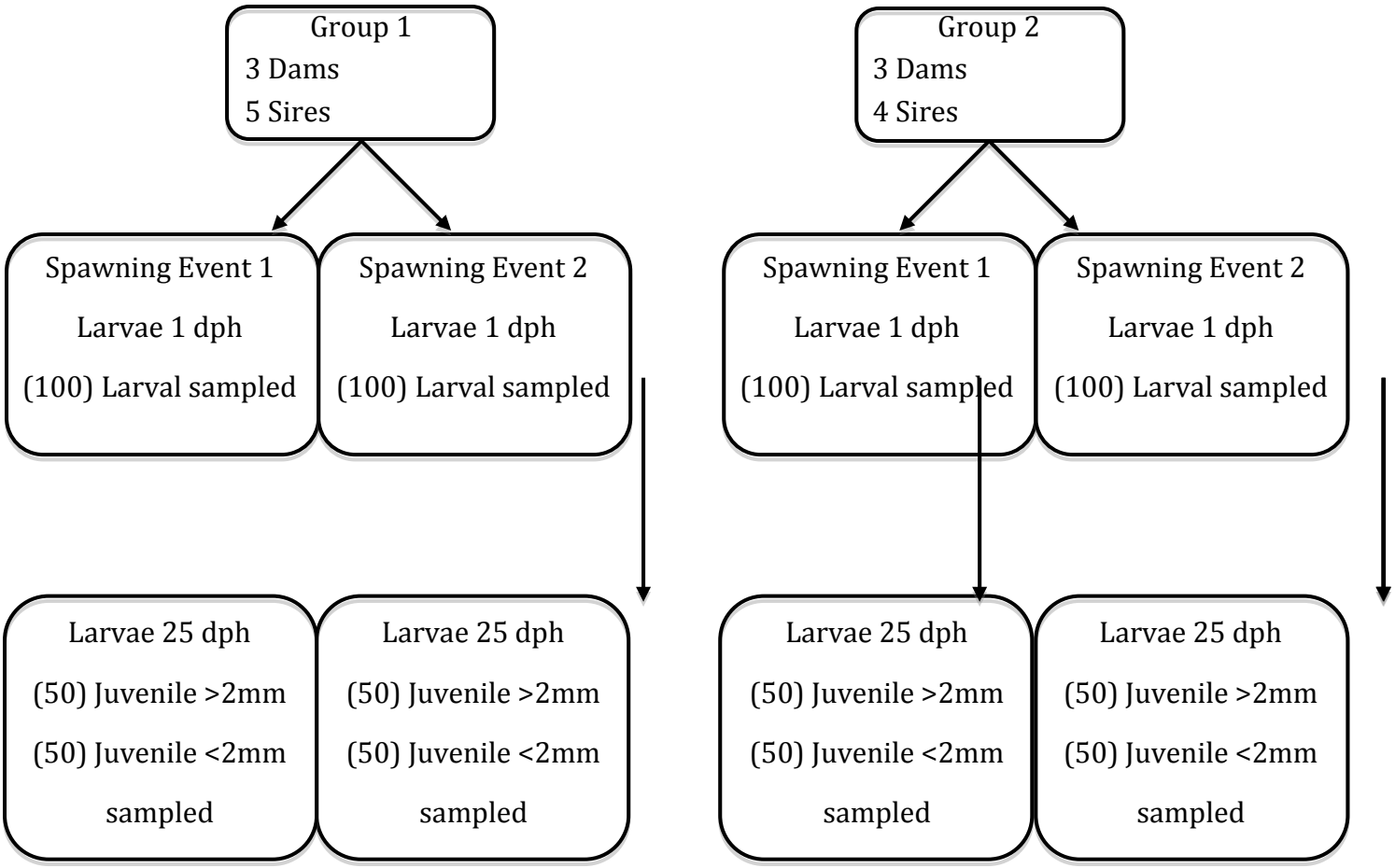

Fig. 1. Sampling protocol, mating scheme and Larvae/ Juvenile sampling used in the YTK hatchery.

2. 3. Larval Sampling

The sampling carried out from the two breeding groups is summarized in Fig. 1. At the time of sampling, 100 1-dph larvae from each breeding group and each spawning event were randomly harvested and stored in 99.9% ethanol at 4^0C until analyzed. Larvae were then cultured as described above to 25-dph, after which they were they were graded through a 2mm grader. 50 25-dph larvae from each breeding group and

spawning event were randomly sampled from each size class (> 2mm - Large and <2mm - Small). The sampled 25-dph larvae from each size are then stored in 99.9% ethanol at 4 ^{0}C until processed.

2. 4. DNA Extraction and Microsatellite genotyping

The DNA was extracted individually from the whole 1-dph larvae (~4-5mm TL) or the caudal fin from 25-dph larvae using the salting out protocol. Six previously published microsatellite loci for the genus *Seriola* (Nugroho and Taniguchi, 1999; Ohara *et al*., 2003; Renshaw *et al*., 2007) were successfully amplified and genotyped in preliminary trials using the broodstock using polymerase chain reactions (PCR). All 6 loci were found to be sufficiently variable in the broodstock to be use in assigning parentage to offspring. PCR reactions contained one μl of DNA, 0.5 units of buffer, 25 mM of $MgCI_2$, 200 μM of dNTPs, 0.05 units of BSA, 10 μM of each primer and 0.05 units of *Taq* DNA polymerase, in a total volume of 5 μl. The PCR conditions included a multiplex reaction at touch down 59 (TD59) (Sdn06, Sdu32, Sequ58 & Sequ77) and a duplex reaction at touch down 55 (TD55) (Sdn07 & Sequ38) with an initial denaturation at 94 ^{0}C for 3 mins, denaturation at 94 ^{0}C for 20 s, annealing at 59 ^{0}C (TD59) and 55 ^{0}C (TD55) for 45 s, extension at 71 ^{0}C for one min for 4 cycles, then denaturation at 94 ^{0}C for 20 s, annealing at 51 ^{0}C (TD59) and 47 ^{0}C (TD55) for 45 second, extension at 71 ^{0}C for one min for 31 cycles and a final extension step at 71 ^{0}C for 4 mins then cooling to 14 ^{0}C. Microsatellites genotyped in this study and their specific PCR conditions are given in Table 1. PCR products were electrophoresesd through a 6% polyacrylamide sequencing gel and visualized by autoradiography. Fragment analysis was performed by the Flinders Medical centre

ABI sequencing facility using the ABI 3130. Allele sizes were then scored using the GeneMapper 4.0 software.

Table. 1. Microsatellites used to genotype two groups of *Seriola lalandi* broodstock involved in a commercial hatchery

Locus	T_a ^{0}C	Primer sequence	Allele size range (bp)	H_E	PCR conditions
Sdn06	52.1	F: GGGTTTGTGCTGTGAGTG	311	0.86	TD 59
		R_a: TCCGTCTGTCTTTTCCTGT			
Sdu32	53	F: CCTGTGAGAGCATTTGGTAT	99-177	1.00	TD 59
		R_c: GTGCTTGTCTCTTCTGTCAT			
Sequ58	52	F: CTGTGTGCTGCTCAAACT	143	0.27	TD 59
		R_d: GCCACATCACATCAGTAC			
Sequ77	55	F: CCTACACATGCACATGAA	192	1.00	TD 59
		R_a: CAAGGCTGATACGTCATG			
Sdn07	48.7	F: CTTGCTCGTCTCATCACCTC	140	0.55	TD 55
		R_b: AACGGCTGGTAAATAGTC			
Sequ38	52	F: CCATTACAATTTGTCTCTC	125	0.93	TD 55
		R_c: CTTATCAACACACGAGCG			

Primers end-labelled with $_a$FAM, $_b$NED, $_c$VIC and $_d$PET fluorescent dyes, T_a ^{0}C = primer annealing temperature, H_E= heterozygosity expected in each loci, TD 59 = touchdown amplification, decrease by 8 ^{0}C, TD 55 = touchdown amplification, decrease by 8 ^{0}C.

2. 5. Parentage Assignment and Statistical Analysis

Parental assignments were performed using "PAPA" (version 2.0) (Duchesne *et al*., 2002). Each progeny was concurrently assigned to a maternal and a paternal parent. The number of offspring contributed by an individual parent to each spawning event was then determined and used to calculate their percentage contribution to the total offspring contributed. Chi squared analyzes were used to determine if contribution levels were significantly different between individual parents within a broodstock group. Similarly, Chi squared analyzes were also used to determine if the contribution levels from 1-dph to 25-dph were significantly different between individual parents within a groups. Effective population size (Ne) was calculated using Ne= 4 (N- 2)/ [(Ks + Vs/Ks) + (Kd + Vd/Kd) – 2] (Vandeputte *et al*., 2004), where N is the number of offspring sampled, Ks and Kd are the mean number of offspring per sire and per dam, and Vs and Vd the variances of sire and dam family sizes. This equation takes into account differences in sample size and the differential levels of contribution made by individual broodstock to the resulting cohort of offspring contributed (Frost *et al*., 2006). Inbreeding value is calculated using the $\Delta F = 1/2Ne$.

3. Results:

3. 1. *Differential broodstock contribution*

3. 1. 1. Group One (1-dph larval)

In "Pooled spawning event one", parentage assignment showed different levels of contributions to the larvae by each of the three dams and five sires (Fig. 2). Contributions were significantly differentiated between sire ($P<0.001$), not between dams. Dam 2 and Sire 1 did not contribute to the overall larvae sampled. The main

contributions were from Dam 1 and Dam 3. Out of four sires that contributed to the sampled larvae, two were the main contributors, with Sire 3 contributing to more than 50% of the larvae. N_e for this spawning event was found to be 3.6, compared to the census population size of 7.5.

In “Pooled spawning event two” the number of contributing dams was reduced from that observed in “Pooled spawning event One”, with only one of the three potential dam contributing to the overall larvae sampled (Fig. 3). The pattern of sire contribution also has changed from Sire 2 & 3 as main contributors in “Pooled spawning event One” to Sire 2 & 4 in the second spawning event. Similarly, Sire 1 did not contribute to the overall larvae sampled. Larvae contributions were significantly differentiated between sires ($P<0.001$). N_e for this spawning event was found to be 2.2, compared to the census population size of 7.5.

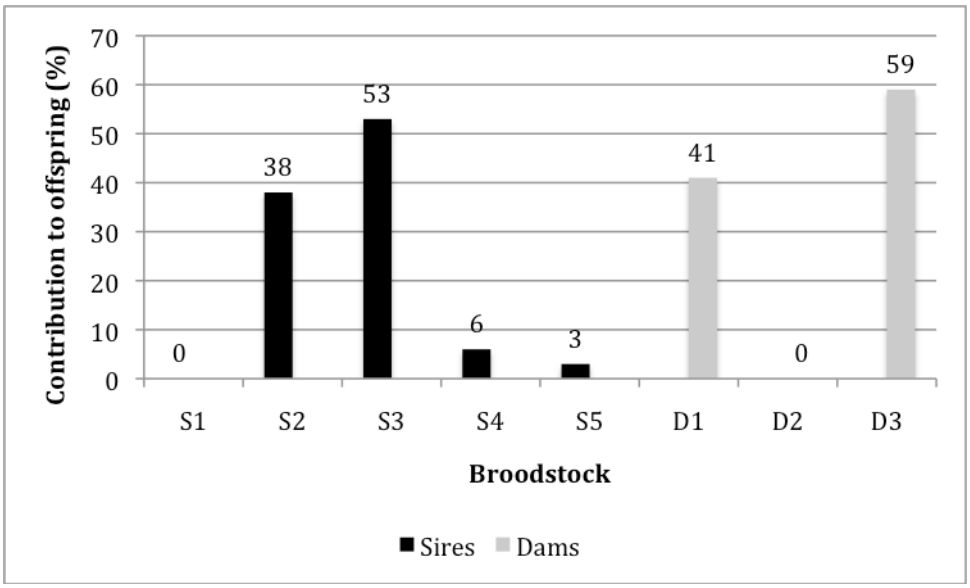

Fig. 2. Contribution of broodstock Group 1 to sampled larvae in pooled spawning event one. Contributions were significantly differentiated between sires (P<0.001), not between dams.

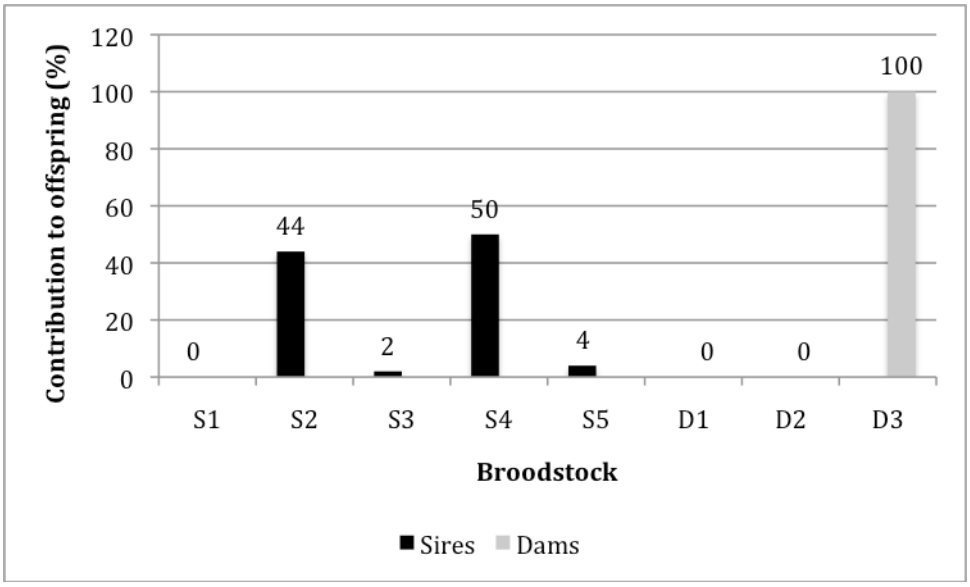

Fig. 3. Contribution of Group 1 broodstock to sampled larvae in pooled spawning event two. Contributions were significantly differentiated between sires (P<0.001).

3. 1. 2. Group Two (1-dph larval)

In “Pooled spawning event one”, genotyped larvae demonstrated that all sires and dams were contributing to the sampled larvae, with the exception of the Dam 2 (Fig. 4). Parental contributions were significantly differentiated between dams (P<0.001) and between sires (P<0.001). The main contribution was from the Dam 3, with 97% of the all larvae sampled from this dam. While all sires have contributed to the gene

pool, two sires (Sires 1 and 2) have contributed to 78% of the sampled larvae. N_e for this spawning event was found to be 2.5, compared to the census population size of 6.9.

In “Pooled spawning event two”, all dams and sires have contributed to the larvae sampled (Fig. 5) as compared to “Pooled spawning event one”, where only two of the three potential dam were contributing to the larvae sampled (Fig 4). While all dams have contributed to the gene pool, the main contributing dam was Dam 3 with 96% of the overall larvae. The pattern of sire contribution changed from Sire 1 and 2 as main contributors in “Pooled spawning event One” to fairly equal contribution from all sires. Parental contributions were significantly differentiated between dams ($P<0.001$), but not between sires. N_e for this spawning event was found to be 2.7, compared to the census population size of 6.9.

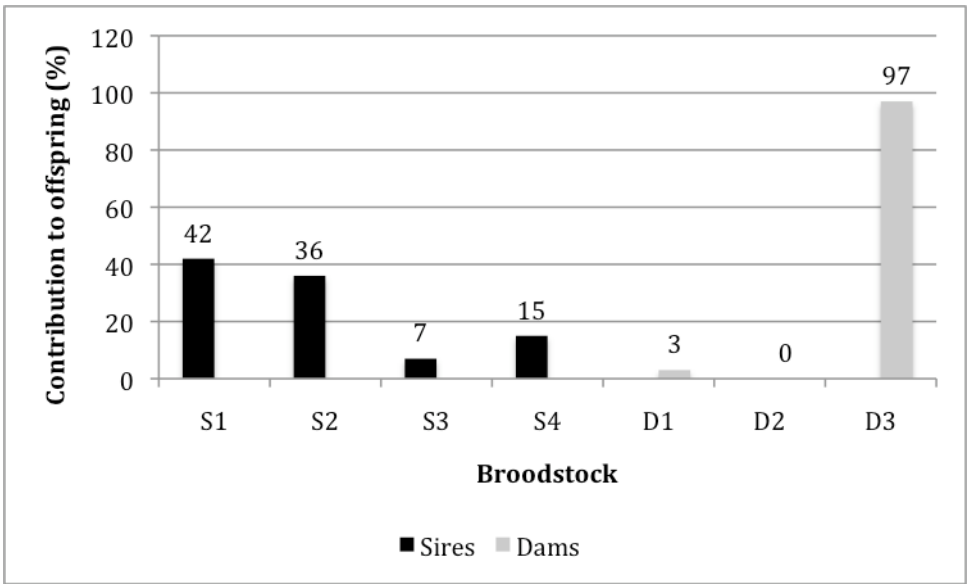

Fig. 4. Contribution of Group 2 broodstock to sampled larvae in pooled spawning event one. Contributions were significantly differentiated between sires (P<0.001) and between dams (P<0.001).

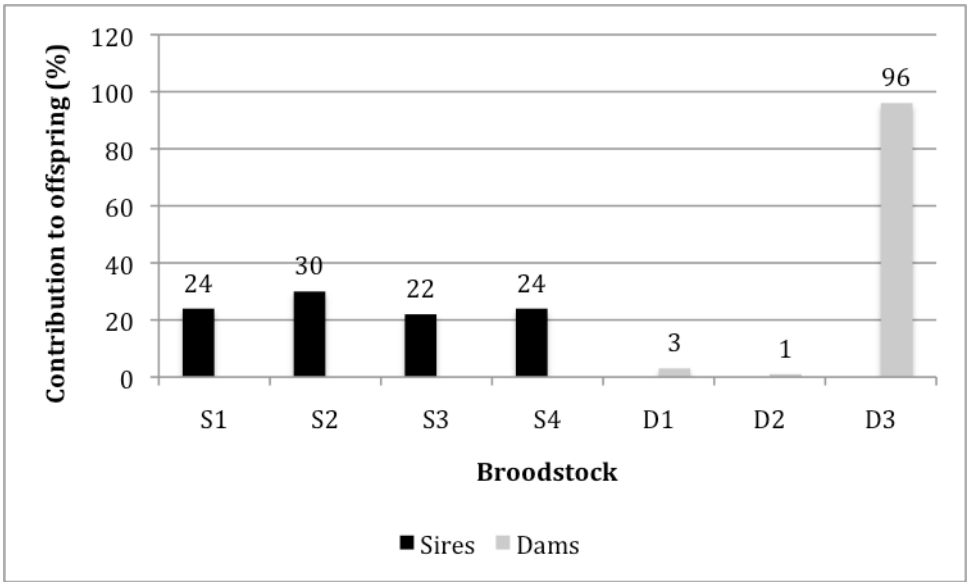

Fig. 5. Contribution of Group 2 broodstock to sampled larvae in pooled spawning event two. Contributions were not significantly differentiated between sires, but significantly differentiated between dams (P<0.001).

3. 2. *Differential Survival*

The level of mortality in the larvae culture was consistently ~ 80% throughout the four pooled spawning events.

3. 2. 1. Group One (25-dph juvenile)

The level of broodstock contribution to juveniles in the “Pooled spawning event one” was found to be changed from that initially observed in the larvae from the same spawning event (Fig. 2). Through hatchery process, where larvae undergo metamorphosis to juvenile, Sire 4 has lost his contribution and a reduction of 25% was observed in Dam 1 contribution. This reduction in Dam 1 contribution was compensated through raising Dam 3 contributions from 59% to 84% (Fig. 6). The differences between contributions at the larval and juvenile stages were found to be significant both between dams ($P<0.001$) and between sires ($P<0.001$). The N_e after the hatchery process also decreased by 25% from 3.6 to 2.7.

Contribution to juvenile in “Pooled spawning event two” was discovered to have changed slightly from that initially observed in larvae (Fig. 3). Due to differential survival the small contribution made by Sire 3 to the larvae was lost by at the juvenile stage there was a reduction of contributions from both Sire 4 and 5 compensated through rising Sire 2 contribution by 17% from 44% to 61% (Fig. 7). The differences between contributions at the larval and juvenile stages were also found to be significant both between dams ($P<0.001$) and between sires ($P<0.001$). The N_e after the hatchery process reduced slightly from 2.2 to 2.1.

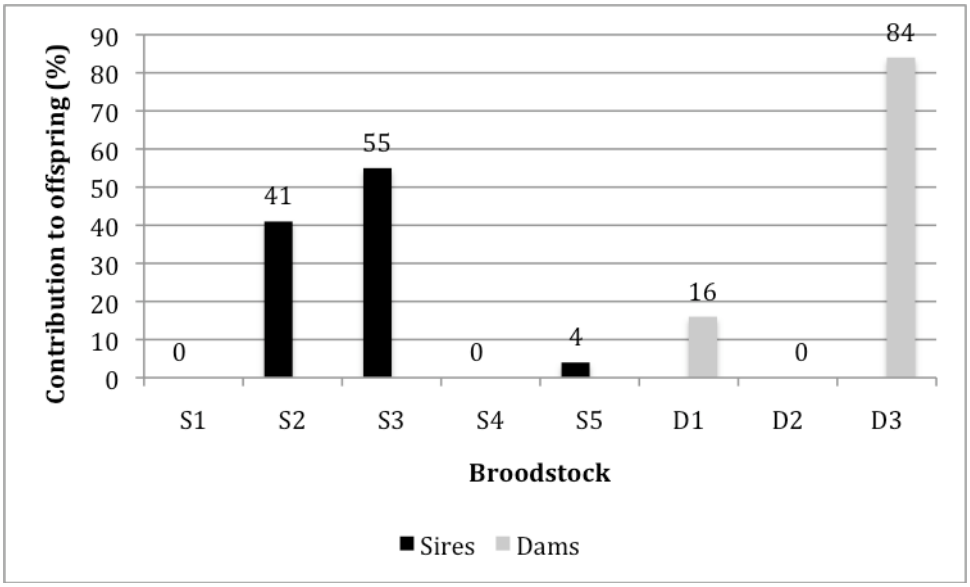

Fig. 6. Contributions of Group 1 broodstock to sampled juveniles in pooled spawning event one. Contributions were significantly differentiated between sires ($P<0.001$) and between dams ($P<0.001$).

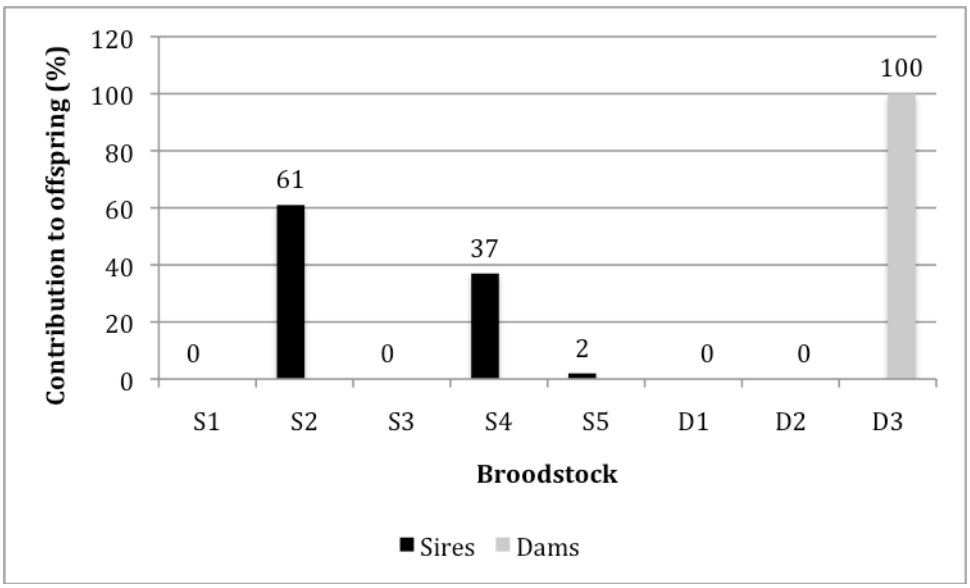

Fig. 7. Contributions of Group 1 broodstock to sampled juveniles in pooled spawning event two. Contributions were significantly differentiated between sires ($P<0.001$) and between dams ($P<0.001$).

3. 2. 2. Group Two (25-Dph juvenile)

The level of broodstock contribution to "Pooled spawning event one" have changed from that initially observed in larval stage. Through the hatchery process, Sire 3 has lost his contribution to the juvenile stage and a reduction in contributions from Sire 2 and 4, were compensated by increase in the Sire 1 contribution by 17% (Fig. 8). Similarly, there was also a 27% reduction of contribution from Dam 3 that was then compensated through increase in the Dam 1 contribution to 30%. The difference between estimated contributions at the larval and juvenile stages were found to be significant both between dams ($P<0.001$) and between sires ($P<0.001$). The N_e after the hatchery process increased from 2.5 to 3.1.

Contribution to juvenile stage in "Pooled spawning event two" have changed from that initially observed in larval stage. Through the hatchery process, a total of 23% reduction of contributions from Sire 2 and 3, were compensated by increase in the contribution of Sire 1 and 4 by 18% and 5%, respectively. The loss of contributions from Dam 2 and 3 were subsequently compensated by increase in the Dam 1 contribution by 17% (Fig. 9). The difference between estimated contributions at the larval and juvenile stages were also found to be significant both between dams ($P<0.001$) and between sires ($P<0.001$). The N_e after the hatchery process increased from 2.7 to 3.4.

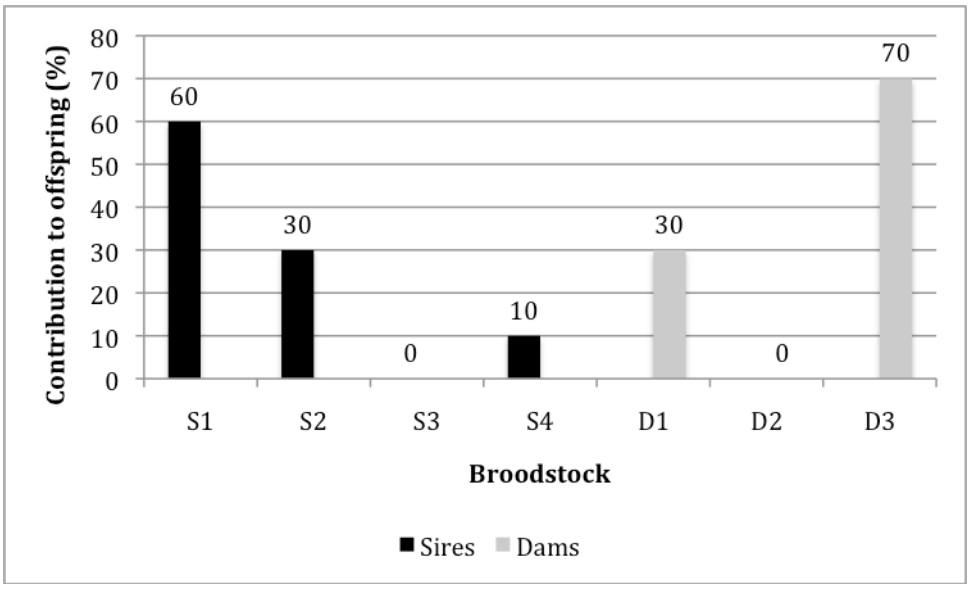

Fig. 8. Contributions of Group 2 broodstock to sampled juveniles in pooled spawning event One. Contributions were significantly differentiated between sires (P<0.001) and between dams (P<0.001).

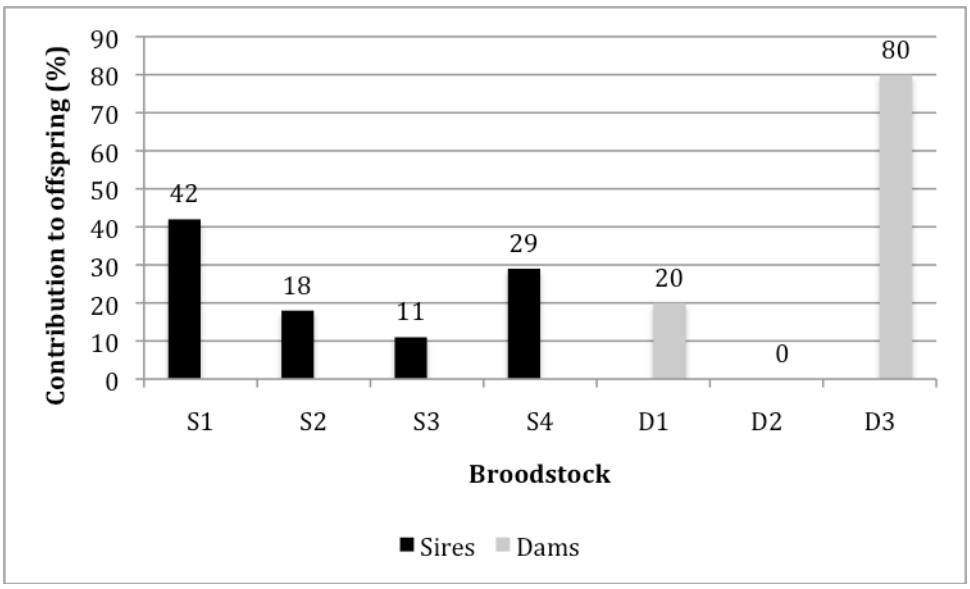

Fig. 9. Contribution of Group 2 broodstock to sampled juveniles in pooled spawning event two. Contributions were significantly differentiated between sires ($P<0.001$) and between dams ($P<0.001$).

Table. 2. Compilation of all spawning event N_e and ΔF for both groups

Group	Event	Day	**N**	**N_M**	**N_F**	**N_e**	**C N_e**	**ΔF (%)**	**CΔF**
1	1	1	8	4	2	3.6	7.5	13	7
1	1	25	8	3	2	2.7	7.5	19	7
1	2	1	8	4	1	2.2	7.5	23	7
1	2	25	8	3	1	2.1	7.5	23	7
2	1	1	7	4	2	2.5	6.9	20	7
2	1	25	7	3	2	3.1	6.9	16	7
2	2	1	7	4	3	2.7	6.9	18	7
2	2	25	7	4	2	3.4	6.9	14	7

N= total number of broodstocks in the group, N_M= number of contributing sires, N_F= number of contributing dams, N_e= Actual effective population size, CN_e= Census effective population size, ΔF= Inbreeding per generation, CΔF= Census inbreeding value per generation.

On the whole, the parental contributions from both broodstock groups show that some broodstock contributions will be lost through the hatchery process. In-addition, the sampling of larvae and juveniles in two separate events would enable the capture of more families' contribution from the existing broodstock population than a one off collection. Furthermore, the actual N_e for both spawning event in either group was >50% lower than the census N_e. As a result the estimated rate of inbreeding based on the actual N_e would be two to three times higher than the census inbreeding value.

4. Discussion:

Prior research has documented that parentage assignment of offspring under commercial hatchery conditions using microsatellite markers is a critical step towards monitoring the effects of aquaculture hatchery practices that could be detrimental to the long-term viability of selective breeding programs (Norris *et al*., 2000; Asahida *et al*., 2004; Borrel *et al*., 2004; Rodzen *et al*., 2004; Vandeputte *et al*., 2004; Fessehaye *et al*., 2006; Frost *et al*., 2006; Brown *et al*, 2005; Castro *et al*., 2007; Herlin *et al*., 2007; Horreo *et al*., 2008; Gheyas *et al*., 2009). The major difficulty in implementing a selective breeding program in pool spawning species is to maintain pedigree information (Frost *et al*., 2006) and manage the loss of genetic variability within the population (Norris *et al*., 2000). Past research on parental assignment in pooled spawning of fish in hatcheries reveals differential genetic contributions, differential survival of larvae to juvenile through the hatchery process (Frost *et al*., 2006) and unequal contribution of parents to offspring (Fessehaye *et al*., 2006; Dupont-Nivet *et al*., 2006; Herlin *et al*., 2008; Blonk *et al*., 2009), which would all contribute to reductions in N_e. Our study has confirmed trends observed in earlier studies mentioned above are also found with YTK.

In YTK, our data revealed that the initial level of genetic contribution by broodstock to two pooled spawning events was skewed and the effective group sizes were much lower than predicted by corresponding census group sizes. Moreover, results showed that further loss of genetic diversity from that initially sampled in 1-dph larval occurred due to differential survival of families throughout the larviculture process in Group 1. However, the differential survival of families through the larviculture process for Group 2 revealed an increase in the N_e from that initially sampled at 1-dph larval. The higher N_e in Group 2 is due to the fact that the contribution was more

equally distributed between two dams (Fig. 8 and 9) rather than one dam which contributed the majority of the offspring to 1-day larval (Fig. 4 and 5). This could be due to earlier spawning activity by Dam 1 before egg collection commenced, which would have resulted in the captured of a lower contribution from Dam 1 and higher contribution from Dam 3. The earlier spawned larvae from Dam 1 will then develop and grow faster than the later spawned larvae due to a competitive advantage for older, larger fish by Dam 3 causing the rise in Dam 1 contributions in 25-dph juvenile.

Asahida *et al*. (2004) reported lack of contribution from some dams as the reason for the loss of genetic variability in the Japanese flounder (*Paralichthys olivaceus*). Similarly, Horreo *et al*. (2008) identified a restricted number of female Atlantic salmon contributions to the offspring as the main cause of loss in genetic variation, possibly due to over maturity of some multi-sea-winter females. In Barramundi (*Lates calcarifer*), genetic contributions to offspring by seven sires was found to be skewed, with two sire contributing 74% of the offspring in a mass spawning event and five other sires contributing 26% of the remaining offspring (Frost *et al*, 2006).

Likewise, skewed contributions were observed in the YTK pooled spawning events, with two sires in "Group 1" contributing more than 90% of the offspring for both spawning events. In "Group 2 pooled spawning event one" two sires contributed 78% of the total offspring while the remaining 22% offspring was contributed by the other two sires. Frost *et al*., (2006) suggested that offspring monopolization by one or two males may be a consequence of sperm competition where sperm from several males are shed into a pool of eggs; sperm competition has shown to significantly reduce N_e from expected levels in many fish species (e.g. Pacific salmon, Campton, 2004). Herlin *et al*., (2008) has reported consistently low effective breeding broodstock size

in gilthead seabream, over a given spawning season, which were attributed to a large number of non-contributing fish, particularly amongst males. Fessehaye *et al*. (2006) wrote that this is a striking feature of many animal species in which few males contribute to most of the matings, while the rest have little or no success. However, in "Group 2 pooled spawning event two" all sires have contributed to the gene pool although level of contribution in each spawning event were different.

Compared to sire contribution, one dam has contributed the majority of the offspring except for "Group 1 pooled spawning event one" where there were two contributing dams. This could be due to the fact that the breeding was carried out towards the end of the normal spawning season and some females may have already ceased spawning or be suffering from reduced fecundity. Herlin *et al*., (2008) reported that sampling of larvae three weeks after the peak spawning season resulted in only 23% of female contributing to the offspring sampled in the Atlantic cod.

The observed N_e was less than 50% of the census values. This finding is common with most commercial marine finfish hatcheries; evidence of low N_e in pooled spawning tanks has also been reported in both gilthead and red seabream (Herlin *et al*., 2008). Given that the minimum targeted N_e for long term management of inbreeding is suggested to be 100 (Borrell *et al*., 2007), both estimated and census N_e (Table. 2) are low and would greatly reduce genetic variance and increase inbreeding in the breeding population if offspring were to be used as future broodstock.

To overcome the limitations of unequal broodstock contribution, hormonal induction with LHRHa could be used to synchronize spawning. This may maximize the contribution from all broodstock and increase N_e and create the opportunity for establishing more families. However, the use of hormonal induction practices while

generally reliable, does not always result in successful spawning (Frost *et al*., 2006). Besides synchronization of spawning with hormone treatments, controlling matings by stripping eggs and sperm, then dividing the eggs into equal aliquots and fertilizing each aliquot with sperm from single male would increase potential N_e (Gheyas *et al*., 2009). This approach would then eliminate sperm competition between males and ensure all males contribute equally. Hence, development of technology for handling YTK to facilitate strip spawning and single pair mating should be investigated. However as far as this technique has yet to be been developed for handling YTK broodstock 'natural' mating in groups is currently the only viable option. It is likely that spawning of individual broodstock will be maximized during the peak of the spawning season and thus the retention of progeny to provide future broodstock should be carried out during the peak of the spawning season so as to ensure receiving more genetic variation in the offspring (Asahida *et al*., 2004).

Regardless of the age of sampling, the sampling of two spawning events in each group has facilitated capturing of more genetic variability and the establishment of more families than is possible from a single spawning event (Table 2). Blonk *et al*. (2009) also reported that sampling from limited number of spawning events would obviously results in lower genetic diversity. Hence, it would be advisable for broodstock managers to retain offspring from different spawning events for culture and subsequent breeding. Above all, more broodstock need to be recruited into the hatchery to increase the N_e and generate more families for the breeding program.

Our result showed evidence for differential larval to juvenile survival, especially in Group 2. The major factors influencing the survival and growth in fish include culture environment, nutrition, cannibalistic behavioral and family genotype. In culture environments the fluctuation or alteration of any of the water quality parameters (e.g.

water temperature, ammonia toxicity, pH and dissolved oxygen level) can result in a drastic increase of mortality. Furthermore, throughout hatchery processes there are critical stages that would result in high mortality (e.g. the commencement of exogenous feeding, swim bladder inflation and the transition from livefeed to artificial diet). The survival of larvae from day 1 to day 25 in this study (20%) was higher than that commonly observed in commercial hatcheries where average survival is approximately 10%. It is therefore likely that differential survival would be further exacerbated in a commercial hatchery system.

The significant changes in broodstock contribution from day 1(Fig. 4 and 5) to day 25 (Fig. 8 and 9) for the two spawning event in breeding Group 2, including a large increase in frequency of offspring from Dam 1 that could be due to faster developing larvae from one female within Group 2. Which could be further explained due to early spawning activity by Dam 1 and thus the failure to capture it's full contribution as the commencement of egg collection was only conducted near dusk, which then captured the left over contribution from Dam 1 while the majority of the contribution could be flushed out before actual egg collection commenced. Logically, then the larvae developing from the Dam 1 could be faster developing larvae due to their slightly advanced age and greater competitive ability compared to larvae derived from Dam 3 as a result of competition for food, space and cannibalism of the smaller larvae from Dam 3 that would reduce the contribution in Dam 3 as shown in (Fig. 8 and 9).

Asahida *et al.*, (2004) suggested that the practice of collecting eggs spawned in a single night, would dramatically reduce the number of the contributing females, especially when only a limited number of collections are taken. By setting up the egg collection nets in place throughout the spawning period may help to capture full contribution from all dams. However, even this would not ensure equal contributions

of families to pools of future broodstock. To counter the problem of differential mortality, it would be very helpful to maintain families (only really identifiable as such if achieved through individual pair mating or artificial spawning and fertilization) separately through early larviculture until mortality has slowed and then pool them in equal proportions to ensure that families are retained through to adulthood.

In order to collect pedigree information in the pooled spawning event, without using microsatellites, offspring from different family groups must be isolated and reared together until they are large enough to be tagged. This approach is both labour intensive and expensive and introduces environmental effect common to full-sib groups, which may confounded the genetic effect (Norris *et al*., 2000). Given that facilities in commercial hatcheries are also limited, in order to overcome these problems increasingly microsatellite loci have been applied as genetic markers for parentage analysis to construct pedigrees. Our genotype result showed that six microsatellites could be used to for effective parentage assignment enabling the identification of critical periods in early YTK hatchery production where the genetic bottlenecks may occur. Increased awareness of the potential for significant losses of genetic diversity and an increased understanding of where the genetic diversity could be lost through the hatchery process can be used to develop improved hatchery techniques that maximize the N_e.

5. Conclusion:

In conclusion we have identified six microsatellites that can be used to assign offspring to the parent broodstock in YTK. Furthermore we identified that the current

mating structure will have the potential to significantly affect the maintenance of genetic diversity in YTK. Particularly, the differential contribution of broodstock to the larvae harvested from the tank and the loss of some parental contributions between 1-dph larvae and 25-dph juveniles and the changes in the frequency of parental contributions resulting from high mortality and differential survival. The result from this study also shows that current N_e is small and greatly reduces genetic variance in the breeding population to an unacceptable level based on the existing broodstock numbers. Hence, the current hatchery structure is not appropriate to the long-term viability of genetic management in a closed hatchery stock or the establishment of a domestication or genetic improvement program. Overall improvements can be achieved by focusing on equalizing the contribution from all broodstocks to increase $N_{e,}$ to minimize the impact of differential family survival during early stages of culture and increase the size of breeding groups. Above all it will be necessary to utilize more broodstock to generate families that will increase N_e for establishing a future breeding program in YTK.

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8. Appendix:

Six microsatellites genotypes for all broodstocks in both group one and two in the hatchery.

S/no	Tube S/no	Group	Sex	Sequ77	Sdn06	Sdu32	Sequ58	Sequ38	Sdn07
1	BY	1	F	163173	299327	113127	144170	123137	140140
2	B200	1	F	157175	329339	135137	148148	105127	140142
3	Or118	1	F	171181	323339	125127	146146	129131	150150
4	W24	1	M	185191	317333	121135	150150	119129	140140
5	W13	1	M	177187	321327	115119	144144	111131	140144
6	W28	1	M	163189	299331	123129	188190	113127	140140
7	19699	1	M	155165	299299	117121	150150	131133	140140
8	25108	1	M	155173	327329	121123	150150	129133	140140

S/no	Tube S/no	Group	Sex	Sequ77	Sdn06	Sdu32	Sequ58	Sequ38	Sdn07
1	Or08	2	F	147181	299323	119133	146146	129129	140140
2	Or09	2	F	163167	325335	121125	162162	123125	140142
3	Or28	2	F	175183	299317	113127	146146	133137	140142
4	Y250	2	M	141163	331331	129137	152152	113131	140142
5	Y249	2	M	177181	321327	117123	150150	113117	140142
6	KFS01	2	M	153185	321331	117133	152164	115125	144150
7	KF402	2	M	163175	331343	111115	152156	121135	140142